Mechanisms of Development 144 (2017) 71–80

Contents lists available at ScienceDirect

Mechanisms of Development

journal homepage: www.elsevier.com/locate/mod

Review

The balancing roles of mechanical forces during left-right patterning and asymmetric morphogenesis

Rita R. Ferreira, Julien Vermot *

Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France
Centre National de la Recherche Scientifique, UMR7104, Illkirch, France
Institut National de la Santé et de la Recherche Médicale, U964, Illkirch, France
Université de Strasbourg, Illkirch, France

ARTICLE INFO

Article history:
Received 1 August 2016
Accepted 3 November 2016
Available online 5 November 2016

ABSTRACT

Left-right patterning and asymmetric morphogenesis arise from a complex set of molecular and cellular interactions that are particularly dynamic and associated with mechanical forces. How do mechanical forces translate into tissular asymmetries? Are these forces asymmetrical *de novo*, or do they build up from pre-existing asymmetries? Advances in developmental genetics, live imaging and cell biology have recently shed light on the origins of mechanical forces generated at the cell scale and their implication in asymmetric patterning and morphogenesis is now emerging. Here we ask when and how, molecular asymmetries and mechanical forces contribute to left-right patterning and organ asymmetries.

Contents

1. Introduction

Mechanical forces are ubiquitous and can modulate the developmental program of plants and animals (Mammoto and Ingber, 2010; Mirabet et al., 2011). Mechanical forces are influent in many steps of embryonic development, from gastrulation to organogenesis (Hamada, 2015; Heisenberg and Bellaiche, 2013; Mammoto and Ingber, 2010). Gastrulation (Behrndt et al., 2012; Farge, 2003; Hiramatsu et al., 2013; Maitre et al., 2012), kidney morphogenesis (Kramer-Zucker et al., 2005), inner ear and otolith formation (Colantonio et al., 2009; Wu et al., 2011), neuron migration (Sawamoto et al., 2006), cardiovascular development (Boselli et al., 2015; Freund et al., 2012; Peralta et al., 2013), haematopoiesis (Pardanaud and Eichmann, 2009), and left-right symmetry breaking (Nonaka et al., 1998) are all mediated by mechanical stresses and force mediated signaling (Zhang and Labouesse, 2012). Prominent mechanical forces-related diseases include cancer (Fernandez-Sanchez et

* Corresponding author.
E-mail address: julien@igbmc.fr (J. Vermot).

http://dx.doi.org/10.1016/j.mod.2016.11.001

al., 2015; Fernandez-Sanchez et al., 2010), ciliopathies (Hildebrandt et al., 2011) and cardiovascular diseases such as atherosclerosis (Hahn and Schwartz, 2009).

Despite the long recognition of the importance of mechanical forces in development, an understanding of how mechanical forces impact development has, until recently, remained elusive. Technological advances in recent years have allowed researchers to study the effects of physical forces on cell behaviors at unprecedented resolution (Ladoux et al., 2016; Lecuit et al., 2011). The results of these studies have led to a paradigm, where in its most extreme form, holds the idea that physical forces, independently of gene expression, can affect tissue development and growth by directly modulating cell behavior (Savin et al., 2011). Mechanical forces have also been shown to act as a key component in the coordination of cell behaviors at the tissue scale, in particular during tissue folding (Striedter et al., 2015). As a consequence, it is now clear that mechanical forces constitute an essential element in multiple aspects of the morphogenetic program (LeGoff and Lecuit, 2016; Zhang et al., 2010).

Forces can be sensed at the molecular and cellular scale through mechanosensitive proteins (Vogel and Sheetz, 2006). A major focus of research is now to define the molecules and signaling pathways associated with mechanotransduction and work from many different fields has now shown that pressure-sensitive membrane proteins, cytoskeletal elements, and extracellular matrix (ECM) components can participate in the interchange between mechanical forces and biochemical signals at the cellular scale (Mammoto et al., 2012; Vogel and Sheetz, 2006). Although much has been done in the study of biomechanical signaling at the cellular scale, the effects of forces at a tissue scale level have emerged only recently (Grill, 2011; Lecuit et al., 2011; Mammoto and Ingber, 2010). The field strongly benefits from concepts and formulation developed by physicists, which promoted the identification and quantification of the relevant forces through unifyed approaches (Grill, 2011). Recent advances in cell biology and live imaging are now allowing researchers to directly assess the distribution of tissue forces, thus helping them to have a better view of how mechanical forces can impact development (Sugimura et al., 2016). This, combined with the discoveries of novel mechanosensitive proteins and pathways, are consistently changing our view of how mechanical forces can impact development.

Left-right patterning and asymmetric morphogenesis is one of the most fascinating aspects of developmental biology. Both the symmetry and asymmetries of the body plan require a number of processes that need to be carefully controlled through a genetic program (Capdevila et al., 2000; Hamada et al., 2002; Pourquie, 2011). Being asymmetric certainly constitutes an advantage in the process of organ packing and positioning in a restrained space. Accordingly, most of our internal organs are asymmetrically positioned within the body cavity. Recent studies in the field of the left-right signaling and asymmetric tissue morphogenesis are now clarifying and reinforcing the interest in the field of mechanical forces and morphogenesis. Examples of tissue asymmetry can be seen in heart tube loop, brain folding, airway branching (Yashiro et al., 2007) and gut looping (Savin et al., 2011). Here, we review the molecular and sub-cellular basis of mechanical and biochemical pathways activated during left-right patterning and asymmetric morphogenesis. Throughout the review, we discuss the potential mechanosensors involved and the mechanical forces generated at cellular and tissue scale.

2. Left-right symmetry breaking mediated by cilia mediated flow forces

Fluid motion is usually mediated by motile cilia in the body. Motile cilia are organelles that protrude from nearly all vertebrate cells with typical lengths between 3 and 10 μm in growing tissues (Avasthi and Marshall, 2012; Ishikawa and Marshall, 2014; Keeling et al., 2016; Vincensini et al., 2011). In vertebrates, cilia are commonly thought to function as chemical and/or mechanical sensors. Motile cilia move fluids, and in doing so they participate in controlling several key developmental processes, such as chemical gradient formation, biomineralization or tubulogenesis (Cartwright et al., 2009). Left-right (LR) specification in vertebrates occurs in the left-right organizer (LRO), which is defined by a group of specialized cells located within the presomitic mesoderm. The cells delineating the LRO are ciliated and contain motile cilia that generate a slow-moving flow (the nodal flow) involved in the initial step of symmetry breaking (Nonaka et al., 1998) (Fig. 1A). Additionally, an intercellular amplification of the asymmetric signals occurs through genetic feedback mechanism near and around the LRO (Nakamura et al., 2006). The prominent models explaining symmetry breaking within the LRO suggests either an asymmetric chemical gradient (Okada et al., 2005), or that the LRO cells can mechanically sense flow due to a particular type of sensory cilia located in the periphery of the LRO, dictates the asymmetry (McGrath et al., 2003; Tabin and Vogan, 2003). While it is possible that these two mechanisms work together, a number of elements are still lacking for our complete understanding of the process (Pennekamp et al., 2015). Importantly, symmetry breakage occurs even in mutant mice with only two motile cilia (Shinohara et al., 2012). Experimental data using a mutant of the Notch signaling pathway and simulations of fluid flow dynamics in the zebrafish LRO revealed a threshold of approximately 30 motile cilia to get a proper LR symmetry breakage (Sampaio et al., 2014). This suggests that the flow detection apparatus is extremely efficient. When considering the flow velocities generated within the LRO of fish, mice and xenopus (Blum et al., 2009; Blum et al., 2014; Schweickert et al., 2007; Supatto and Vermot, 2011), it appears that they are much lower when compared to other organs - for example, they are 3 to 10 times lower than the hemodynamics generated in the vascular network even at its earliest embryonic stages (Anton et al., 2013; Cartwright et al., 2009; Goetz et al., 2014; Hove et al., 2003; Supatto and Vermot, 2011).

The mechanosensory hypothesis has been favored by the discovery that Trpp2 (PKD2 or polycystic kidney disease protein 2) is key for LR patterning (Field et al., 2011; Kamura et al., 2011; McGrath et al., 2003; Pennekamp et al., 2002; Schottenfeld et al., 2007; Yuan et al., 2015). Trrp2 is a potent mechanosensory protein (Patel et al., 2010; Sharif-Naeini et al., 2010) both in kidney and vasculature (Goetz et al., 2014; Nauli et al., 2003; Nauli et al., 2008) that acts in combination with Pkd1 at the cell membrane. In zebrafish, Trpp2 is necessary for the genesis of asymmetric calcium release around the LRO, which is initiated within cilia (Yuan et al., 2015). Mutant protein of Trpp2 that cannot bind to the membrane cannot rescue Trpp2 loss of function in the LRO and lead to LR symmetry defects (Yoshiba et al., 2012). Trpp2 belongs to the big family of transient receptor potential proteins (TRP) that contain a number of mechanosensitive channels. Yet, Trpp2 is not a 'canonical' stretch sensitive channel and its biology is extremely complex and cell type specific (Giamarchi et al., 2006): it is part of a multiprotein complex involved in transducing Ca^{2+}-dependent information. It localizes to primary cilia of renal epithelial cells, where it seems involved in mechanosensitive transduction signals (Nauli et al., 2003; Pazour et al., 2002; Yoder et al., 2002), but it has been observed at the cell membrane and in the ER. Trpp2 has been shown to inhibit the response of stretch activated cation channels in smooth muscle cells, suggesting that it can modulate mechanotransduction without being a mechanosensor itself (Sharif-Naeini et al., 2009). Recently, the group of David Clapham showed that intraciliary calcium increase is not observed in the mouse LRO in response to flow forces, suggesting that the primary function of TRP channels, including Trpp2, is not to modulate intraciliary calcium in response to cilia bending, and, as a consequence, do not act as mechanosensor in this context (Delling et al., 2016). In that aspect, it is worth mentioning that Pkd2 mutants do not present apparent defects in intracellular calcium levels in the node (Yoshiba et al., 2012). Importantly, Trpp2 frequently acts in combination with other mechanosensitive proteins such as Trpv4 (Du et al., 2014; Heckel et al., 2015; White et al., 2016), Pkd1 (Hanaoka et al.,

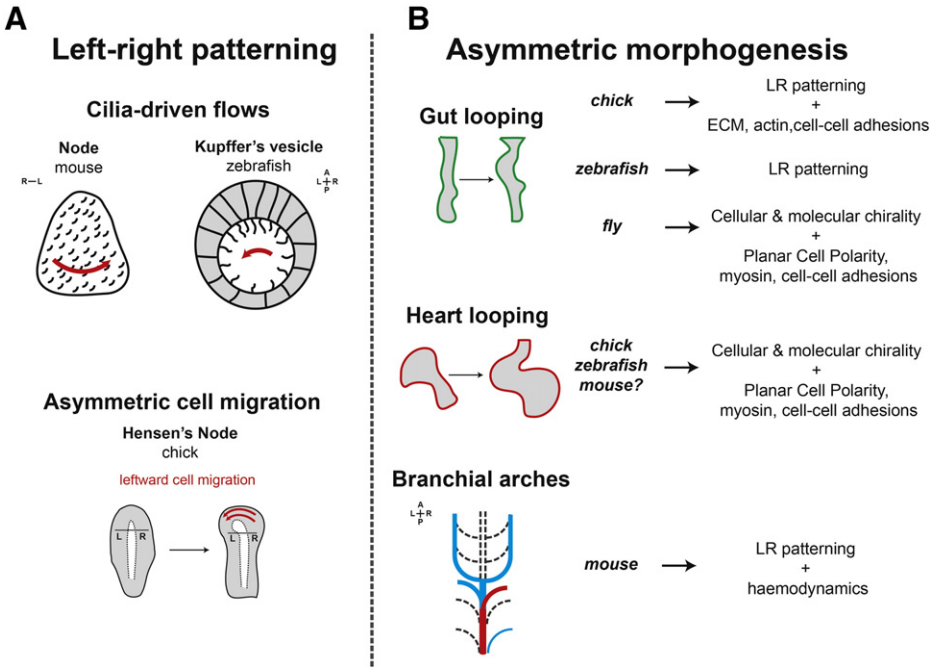

Fig. 1. Summary of left-right patterning and asymmetric morphogenesis: (A) Left-right (LR) specification in vertebrates occurs in LR organizers (top left: mouse Node; top right: zebrafish Kupffer's vesicle; down: chick Hensen's node). The cells delineating the mouse and zebrafish LR organizers are ciliated and contain motile cilia that generate a cilia-driven slow-moving flow (the nodal flow – red arrow) involved in the initial step of symmetry breaking. The chick embryos do not seem to rely on cilia-driven flow but on asymmetric cell migration to break LR symmetry. During development, the cells around the Hensen's node (bottom left) experiences an asymmetric cell migration with changes in adhesion, thus interfering with the LR patterning of chick embryos. (B) Asymmetric morphogenesis of internal organs in different model systems required not only LR patterning but also depend on cellular and molecular chirality, as well as tissue intrinsic properties such as cell-cell adhesion, cytoskeleton components like actin and myosin, among others. In panel B, we summarize gut and heart looping in the different animal models as well as branchial arch formation in the mouse embryo.

2000; Nauli et al., 2003) or Pkd1l1 (Grimes et al., 2016; Kamura et al., 2011) to mediate the calcium flux. During left-right patterning, the partner of Trpp2 is Pkd1l1, Pkdl1 physically interacts with Trpp2 (Field et al., 2011) and is necessary for LR patterning in mouse and medaka fish (Field et al., 2011; Grimes et al., 2016; Kamura et al., 2011). Additionally, Pkd1l1 might be sensitive to proteoglycan distribution (Superina et al., 2014) and it is suspected that the ECM can alter Pkd1l1 biological activity at the cell surface. All in all, these recent results suggest that TRP channels might act as a mechanosensor in the LRO, yet the mechanism by which they operate remains mysterious. Along the same line, how do cilia sense flow and whether non-ciliary sensory mechanisms are involved in the LR breaking are crucial questions to answer for our understanding of the mechanism of symmetry breaking. When considering the pkd1/trpp2 complex, it seems that the couple Pkd1/Trpp2 also has non-mechanosensitive signaling properties. For example, it looks like Pkd1 can act as a prototypical membrane receptor that concordantly regulates Pkd2 channels and G-proteins in neurons and kidney cells (Delmas et al., 2004). Interestingly, Pkd1 proteins can be activated by potent signaling molecules, such as Wnt ligands (Kim et al., 2016), to activate intracellular calcium signaling. Considering the multiplicity of outcomes trpp2/pkd1 interactions and activations can lead to, a better understanding of the targets of mechanism of action of Trpp2 and Pkd1l1 at the cell membrane and cilia will be key to figure out the mechanism initiating asymmetric gene expression in the LRO. Most importantly, it will help to understand if (and how) mechanotransduction is indeed at work in the LRO.

3. Left-right symmetry breaking mediated *via* asymmetric cell migration and adhesion

A few vertebrate species do not seem to rely on cilia mediated flow (Blum et al., 2009; Gros et al., 2009) but depend on asymmetric cell migration to break left-right (LR) symmetry. Intrinsically, cells are chiral and naturally spread with a chiral order when plated on a dish (Chen et al., 2012; Tee et al., 2015). Additionally, cells tend to migrate with directionality which is dictated by the substrate (Caballero et al., 2015; Comelles et al., 2014) suggesting that cell migration directionality is mechanosensitive. Importantly, the position of the centrosome, which can be considered as the actin and microtubule organizer of the cells, is also dictated by cellular force distribution in cell culture (Farina et al., 2016; Pitaval et al., 2010). A good example of how asymmetric cell migration and adhesion play a role in the patterning of embryos during development concerns the cell movements around the Hensen's node. It was shown that asymmetric cell rearrangements take place within the node of chick embryos, thus creating a transient leftward movement of cells around it (Cui et al., 2009; Gros et al., 2009), which later stops (Fig. 1A). The migration of cells away from the midline to the left side consequently deform the shape of the node. Thus, these leftward movements lead to the asymmetric expression of *shh* (Sonic Hedgehog) and *fgf8* (fibroblast growth factor 8). *Shh* is initially expressed bilaterally at the rostral side of the node and later becomes restricted to the left. Moreover, the *fgf8* bilateral expression in the primitive streak results in an asymmetric expression on the right side of the node (Cui et al., 2009; Gros et al., 2009). To understand how the leftward movement of cells occurs in the chick, Gros and colleagues (Gros et al., 2009) studied the driving force for cell rearrangements and how they could disrupt it (and which consequences will arise from it). Their work has shown that by impairing the myosin-II pathway, as well as, by physically blocking cell movements in the node, the leftward movement of cells was no longer seen. Likewise, the chick node lost its asymmetrical shape on the LR axis, when compared to control embryos (Gros et al., 2009). In addition to the disruption of the leftward movement of cells, Gros and colleagues have reported the bilateral gene expression of *shh* and *fgf8*, thus suggesting the leftwards cell movements are required to initiate the LR asymmetric expression domains in the chick embryo (Gros et al., 2009). Nevertheless, until recently, there was no answer

for the question of how this transient leftward movement of cells in the chick node was controlled in a time-dependent manner. In other words, it was not clear how the transient movement would stop once the asymmetry has been established. Based on the evidence that N-cadherin is asymmetrically expressed in a time-dependent fashion in the node and its inhibition gives rise to heart misplacement (Garcia-Castro et al., 2000), Mendes and colleagues have recently proposed N-cadherin as a good candidate to stop the transient leftward movements, possibly *via* an asymmetric cell-cell adhesion mechanism (Mendes et al., 2014). This study combined a photoconvertible fluorescent protein (*Kaede*) with *in vivo* microscopy to track single cell movements in the node of the chick embryo, and in this way investigated the migratory behaviors in the node region in response to N-cadherin perturbations. They concluded that N-cadherin is important to stabilize the molecular asymmetries established earlier in the node, so that the correct asymmetric information is transferred to the lateral plate mesoderm (LPM) and the proper asymmetric looping of the heart is achieved (Garcia-Castro et al., 2000; Mendes et al., 2014). Since cadherin proteins are known mechanosensitive proteins (Huveneers and de Rooij, 2013; Ladoux et al., 2010; Lecuit and Yap, 2015; Weber et al., 2012), it would be interesting to assess if the process of asymmetric cell migration is in itself driven by mechanical forces or by the intrinsic cell chirality often observed *in vitro*.

4. Cell contractility and forces associated with left-right organizer formation

Recent studies have identified regulators of the actomyosin cytoskeleton (Wang et al., 2011; Wang et al., 2012) and components of the extracellular matrix (ECM) (Compagnon et al., 2014) as mediators of cell positioning in the zebrafish left-right organizer (LRO) (called Kupffer's vesicle (KV)), important for the breaking symmetry event. The ECM is thought not only to provide a structure to support organs but also to control cell-cell communication, proliferation, differentiation, and migration.

The KV in zebrafish is formed by a group of nearly two-dozen cells, known as dorsal forerunner cells (DFCs), which migrate deep into the embryo through development. These cells undergo a mesenchymal-to-epithelial transition (MET) to form the KV in a vesicle-like structure with a mono-ciliated epithelium (Essner et al., 2005). Several studies confirmed the existence of a cluster of ciliated-cells in the anterior-dorsal (AD) region of the KV (Kramer-Zucker et al., 2005; Kreiling et al., 2007; Okabe et al., 2008), suggesting this higher density of cilia (as a consequence of a higher cell density) can cause the strong directional flow observed in the KV (Kramer-Zucker et al., 2005; Kreiling et al., 2007; Okabe et al., 2008; Sampaio et al., 2014; Wang et al., 2011; Wang et al., 2012) (Fig. 1A).

The knowledge gap about the molecular and cellular mechanisms regulating the asymmetries in cell density within the KV has only recently started to be filled. Wang and colleagues started by proposing a model of cell remodeling that would allow an initially symmetric organ to acquire anterior-posterior (AP) asymmetry. In this model, anterior KV cells would be more tightly packed than posterior cells, as a consequence of the gradient of cell tension in the AP axis (Wang et al., 2011; Wang et al., 2012). They have identified Rock2b (Rho kinase protein) as a key regulator of KV remodeling (Wang et al., 2011). Depletion of the Rock2b-Myosin II pathway resulted in the disruption of the cell cluster in the AD region, changes in cell morphology and impairment of the asymmetric cilia-driven flow, which then impacts the proper establishment of the left-right (LR) axis (Wang et al., 2011; Wang et al., 2012). Making use of mathematical simulations, they proposed a model in which the Rock2b-Myosin II pathway regulates cell–cell interfacial tension during KV remodeling, by regulating cell contractility and cell adhesion (Wang et al., 2012).

More recently, by studying endogenous and ectopically induced KV, Compagnon and colleagues proposed that local differences in the shape of KV ciliated-cells are the result of localized ECM deposition at the surface of the adjacent notochord. This accumulation of ECM would restrict the apical expansion of the lumen-lining KV epithelial cells within the AD region, in response to lumen growth during the development of the KV (Compagnon et al., 2014). In this work, they have shown that laminin and fibronectin strongly accumulate at the axial-paraxial boundary adjacent to the AD region of the KV highly packed with ciliated-cells. Furthermore, interfering with these ECM components result in the impairment of the KV remodeling process important for the breaking of LR symmetry, suggesting that ECM-dependent cell shape changes are critical for KV function (Compagnon et al., 2014).

These studies suggest that a highly regulated organization of the LRO is dependent on cellular forces. Proper modulation of these forces is thus crucial for generating an architectural asymmetry within the organ, thus playing a key role for its function as LRO in zebrafish.

5. Molecular and subcellular chirality in the process of left-right patterning

At the molecular scale, asymmetries of sub cellular components has long been thought to provide the initial asymmetry necessary to initiate an asymmetric gene cascade (Brown and Wolpert, 1990; Levin and Mercola, 1998). Interestingly, this hypothesis fits when considering the molecular architecture of the internal organization of motile cilia, which is chiral (Figs. 2 and 3). The body of the cilium is made of a chiral alignement of microtubule (as well as its basal body) such that the direction of cilia rotation has been proposed to be determined by the structural interaction of their protein building blocks sliding, which has to be chiral as well (Hilfinger and Julicher, 2008). Flow in the LRO can thus been considered as a way to convey the molecular chirality to an asymmetric flow (Levin, 2005). In this model, the obtained cilia mediated flow allows to scale up the molecular asymmetries to the LRO (Fig. 2).

However, the direction of cilia motility is not enough to drive a directional flow. Cilia need to be posteriorly tilted in order to generate a directional flow, and this in turn depends on the proper positioning and orientation of the cilia at the posterior side of cell surface of the LRO (Hashimoto and Hamada, 2010; Supatto and Vermot, 2011). The molecular mechanisms that set this orientation depends on the Planar Cell Polarity (PCP) pathway (Borovina et al., 2010; Hashimoto et al., 2010; Song et al., 2010). This process is dynamic, as it seems that cilia move towards caudal side of the node in response to the PCP (Hashimoto et al., 2010). The gradual posterior positioning of the basal body correlates with increase flow in the node, which suggests that the posterior tilt increases accordingly. Interestingly, it has been shown that the process of cilia positioning in multiciliated cells is force dependent - flow itself has been shown to modulate cilia orientation in brain ependymal cilia (Guirao et al., 2010) and is mediated by Pkd1 and Pkd2 (Ohata et al., 2015). This mechanism, though, does not seem at work in the node where cells are monociliated, since pkd2 mouse mutants do have normal nodal flow (Yoshiba et al., 2012). In addition, exogenous strain polarizes apical microtubules, and align stable components of the PCP pathway orthogonal to the axis of strain in the developing skin of xenopus (Chien et al., 2015). Thus, it seems that oriented tissue strain can play a role in determining the global axis of planar polarity *in vivo* (Chien et al., 2015). It will be interesting to test if tissue strain is oriented in the node and if cilia orientation can be affected as a consequence of strain, and, potentially, mechanotransduction. Furthermore, the mechanism that position the cilium is thought to be microtubule dependent, but the role of actin might have been understudied. The recent discovery that the centrosome also acts as an actin organizer might trigger more effort in that direction of research (Farina et al., 2016).

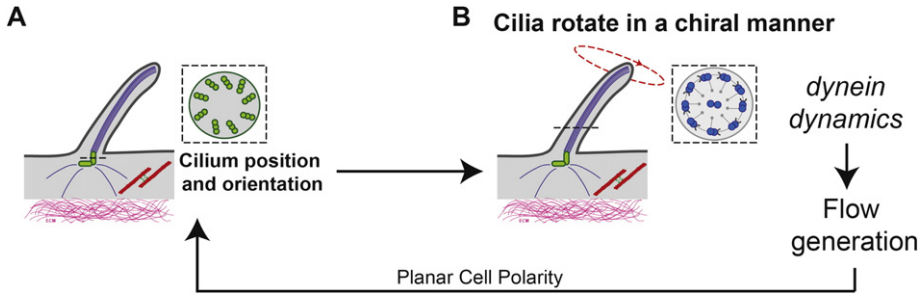

Fig. 2. Cilia position and orientation is important for cilia-driven flow generation in the left-right organizers (LRO): (A) the cilium orientation is initially determined by its basal body (green struture). A cross section on the basal body (inset) shows its nine-fold symmetric organization. The basal body dictates at some extent the degree to which cilia are tilted at the cell surface and thus, could be linked to the correct direction of cilia-driven fluid flow in the LRO. (B) Cilia in LRO rotate in a chiral manner, depending on the way the motor dyneins crosslink with the set of microtubules inside the cilium (inset). Different structures can be found in the motile cilia of the LRO: in the mouse Node, the motile cilia have a typical 9 + 0 structure (absence of central pair), whereas in the zebrafish Kupffer's vesicle the central pair is present (9 + 2 structure as shown in the inset). Cilia rotation set the forces to generating a chiral fluid flow, capable to break the symmetry of many vertebrates. Furthermore, cilia-driven flow generated in connection with the cell cytoskeleton (red:actin and green:myosin), and in addition to an expected contribution of the Planar Cell Polarity pathway, can employ a potent organization on the orientation of the basal body itself.

6. Cell and tissue chirality in the process of asymmetric morphogenesis

The translation of molecular asymmetry into mechanical forces is certainly best known from the work perfomed in cultured cells and, more recently, in *C. elegans* under the impulsion of biophysicists. Their work mainly focuses on the actomyosin network, which corresponds to the key effector of the molecular motors that drive cell shape and migration. Together, this work suggests that the entire actin network at the cellular scale can have a chiral behavior in response to the rotational forces produced at the focal adhesion of the cells (Naganathan et al., 2016; Tee et al., 2015). Recent studies provide new insights on the chiral organization of the actin network (Chen et al., 2012; Naganathan et al., 2014; Tee et al., 2015). Chen and colleagues have shown that when vascular mesenchymal cells grow until confluency on micro-patterned stripes, they align in a constant chiral fashion, which could be disrupted by drug treatments for myosin II or Rho signaling (Chen et al., 2012) (Fig. 3B). Also, for single fibroblasts plated onto round micro-patterns, it was shown that the actin cytoskeleton self-organizes in a chiral pattern, revealing an unusual transition from a radially symmetric pattern to another that is chiral (Tee et al., 2015). Interestingly, the handedness of the chiral pattern can be changed by a single protein, the alpha actinin 1, suggesting that cell chirality can be turned on and off by a single protein (Tee et al., 2015). In another study using *C. elegans*, Naganathan and colleagues quantitatively demonstrated that the generation of active chiral torques by the actomyosin cortex facilitates chiral symmetry breaking along the antero-posterior axis of the embryo (Naganathan et al., 2014). Also, they have shown that active torques are dependent on myosin activity, and can be altered by modulating Rho signaling (Naganathan et al., 2014). Taken together, these studies argue for the idea that chirality of cells and tissues might be dependent on the proper alignment of molecular torques generated by the actomyosin activity. While still early, it is tempting to extend this concept to chiral morphogenetic rearrangements that have been observed at other stages in *C. elegans* development (Pohl and Bao, 2010) and during the first cleavage (Schonegg et al., 2014; Singh and Pohl, 2014).

In summary, interesting hypotheses are now emerging in order to explain the role of subcellular asymmetries at the embryonic scale in organisms possessing a left-right organizer. An intriguing possibility is that cell chirality could be used as an additional element to provide the embryo with handedness, forcing tissue asymmetries independently of the canonical left-right signaling pathway (McDowell et al., 2016) (Figs. 1 and 3).

6.1. Gut looping chirality

One of the most striking examples of asymmetric organ morphogenesis in response to left-right (LR) positional cues (Burdine and Schier,

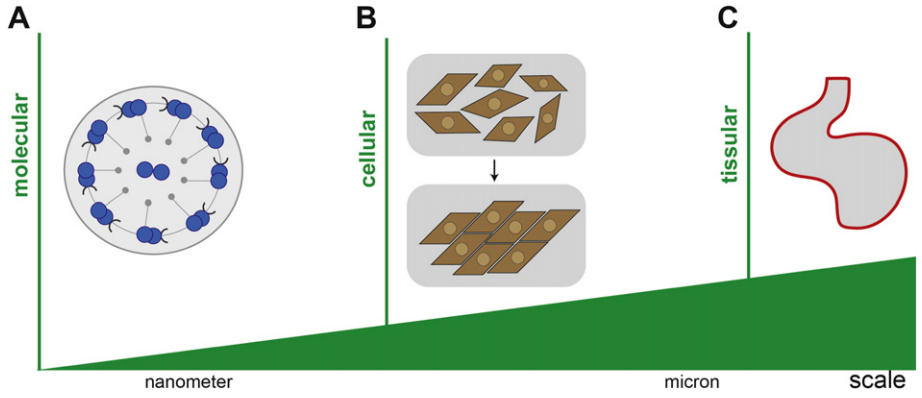

Fig. 3. Different scales of chiral organization: from molecular to tissular level: (A) Cross section of a motile cilium, characterized by an axoneme, which consists of a regular cylindrical arrangement of microtubules (in blue), crosslinked in a chiral fashion by motor proteins called dyneins (in dark grey). Inside the axoneme of the cilium, it is the action of crosslinking dyneins (and other) proteins, that generate internal forces that localy slide the microtubules, leading in turn to the bending of the cilium. (B) At the cellular level, various types of cells are chiral and naturally spread with a chiral order when plated on a dish. The chiral organization of the actin network itself may explain this behavior is then translated to the cell. In this schematics, confluent vascular mesenchymal cells (in brown) grow until confluency on fibronectin plates (in grey), and then align in a constant chiral fashion, in an actin-dependant fashion. (C) An example of an internal organ whose formation and morphogenesis is dependent on cellular and molecular chiralities is the embryonic heart.

2000; Levin, 2005; Tabin and Vogan, 2003) is the gut, in which the liver is positioned on the left side, whereas the pancreas remains on the right side of the body plan. Also the intestine rotates and folds in a complex pattern to facilitate its packing in the abdominal cavity (Horne-Badovinac et al., 2003). In vertebrates, the embryonic gut tube forms the intestines through a characteristic looping after an initial 270° rotation (Savin et al., 2011). Savin and colleagues analyzed the effects of forces at a tissue scale level during the gut morphogenesis in chick embryos (Savin et al., 2011). They have proposed that homogeneous and isotropic forces, which arise from the relative growth between the gut tube and the dorsal mesentery (DM) could be at the origin of the gut loop formation in the chick embryo (Savin et al., 2011). Based on their experimental observations, after physical separation of the DM from the gut, the intestine uncoils into a straight tube, indicating that it was under compression, whereas the unconstrained DM contracts, indicating that was under tension. Thus the gut–DM complex is essential to maintain the mature loops in the gut (Savin et al., 2011). Their theoretical model captured the key properties of the looping patterns, strongly suggesting the gut looping pattern is established by the balance of forces induced by the relative growth between the gut-DM complex (Savin et al., 2011).

Yet, the mechanism that drive asymmetric positioning of the gut is not only mechanical but follows asymmetric cues acting downstream of the canonical left-right pathway. Visceral organs are surrounded by a basement membrane (specialized ECM) that mediates mesoderm-endoderm interactions critical for organogenesis. Work done in chick and mouse (Davis et al., 2008; Kurpios et al., 2008) has shown that the looping direction of the gut is established by modifications both in the ECM and in the adhesion of mesenchymal cells. *In silico* data proposed that mesenchymal cells are more densely packed on the left side, and this can be a consequence of LR asymmetries in both ECM and cell-cell adhesion (Kurpios et al., 2008). These asymmetries are regulated by the asymmetrically expressed *Nodal*-induced transcription factors *Pitx2*, *Isl1* (both left) and *Tbx18* (right) (Davis et al., 2008; Kurpios et al., 2008). Both *Pitx2* and *Isl1* up-regulate N-cadherin activity on the left DM, thus changing the morphology of epithelial cells and increasing the aggregation level of mesenchymal cells on the left DM (Kurpios et al., 2008). Both cell changes promote a tilt in the developing midgut that provides the LR bias needed to later induce the counterclockwise gut rotation, and failure to do so leads to defects in gut rotation (Davis et al., 2008; Kurpios et al., 2008). Even though *Pitx2* has a major role is the gut development, its cellular targets that drive asymmetric morphogenesis are not known. Welsh and colleagues have shown that *Pitx2*-specific effectors mediate Wnt signaling and that Wnt pathway components were asymmetrically expressed according to the LR axis (Welsh et al., 2013). Their work established a link between actin dynamics and cadherin-based junctions, which culminate in the asymmetric cell behaviors seen during gut morphogenesis in chick embryos (Davis et al., 2008; Kurpios et al., 2008; Welsh et al., 2013). Also, Mahadevan and colleagues have shown that the process of arteriogenesis in the DM begins during gut rotation and continues strictly on the left side, and is dependent on the *Pitx2* target gene *Cxcl12*. The same work revealed that gut lymphangiogenesis starts on the left DM, in a process dependent on gut arteriogenesis. Thus, they have proposed that the *Pitx2* LR-pathway drives arterial and lymphatic vessels development in the gut (Mahadevan et al., 2014) (Fig. 1B).

In zebrafish, the gut tube originates from a solid rod of endodermal cells that forms a lumen as the cells polarize (Ng et al., 2005; Ober et al., 2003; Wallace and Pack, 2003). During the looping of the gut, the left and right LPM migrate separately, dorsal and ventrolateral to the gut, respectively (Horne-Badovinac et al., 2003). This asymmetric migration displaces the gut to the left. It occurs specifically within the gut-looping region and requires functional LR gene expression and establishment of epithelial polarity within the LPM (Horne-Badovinac et al., 2003). Mutations that disrupt the epithelial structure of the LPM perturb this asymmetric migration and inhibit gut looping. Asymmetric LPM migration still occurs when the endoderm is ablated from the gut-looping region, suggesting that the LPM can autonomously provide a motive force for gut displacement (Horne-Badovinac et al., 2003). Work from Yin and colleagues gave new insights about the role of the ECM remodeling during the asymmetric migration of the LPM during zebrafish gut looping (Yin et al., 2010). They have shown that a localized reduction of laminin deposition is necessary for the asymmetric cell rearrangements within the LPM, as a consequence of the degradation of the basement membrane at the LMP-gut boundary. Thus, it was revealed that such LPM-ECM interaction is crucial for the asymmetric migration of the LPM during gut-looping (Yin et al., 2010). Hochgreb-Hagele and colleagues continued exploring the role of laminin in this context (Hochgreb-Hagele et al., 2013). Using laminin mutants, they observed that due to the lack of basement membrane at the LPM-gut boundary, some LPM cells escape from the LPM and protrude into the gut. Such cell behavior disrupts the normal communication between the LPM cells and stops the collective migration of the LPM (Hochgreb-Hagele et al., 2013). Therefore, there is now enough evidence supporting the important role of the ECM during the establishment of LR axis during the organogenesis of visceral organs. Furthermore, it is established that the epithelial LPM determines the chirality of gut looping and thus the asymmetric position of the digestive organs in several vertebrates (Davis et al., 2008; Hochgreb-Hagele et al., 2013; Kurpios et al., 2008).

In invertebrates, LR asymmetries can be also observed. Several tissues in the fruit fly *Drosophila melanogaster* display LR asymmetries and chiral morphogenesis, like the brain, Malpighian tubules, genitalia and gut (Geminard et al., 2014). The discovery of the conserved *myosin ID* gene (MyoIC and MyoID) as being a main element of LR asymmetry revealed a novel pathway involving actin cytoskeleton and adherens junctions (Hozumi et al., 2006; Speder et al., 2006). MyoID is a dextral determinant for the orientation of all *Drosophila* LR visceral organs (Hozumi et al., 2006; Speder et al., 2006). Mutants for MyoID show reversed lateralization of the internal organs (Hozumi et al., 2006; Speder et al., 2006; Speder and Noselli, 2007). Furthermore, knockdown of myoID in a specific tissue lead to abnormal LR phenotypes exclusively in the affected tissue, without disturbing the laterality of other organs (Speder et al., 2006). This suggests the existence of additional tissue-specific LR organizers (LRO) that remain to be characterized. Furthermore, in these LROs, it was shown that beta-catenin and DE-cadherins (*Drosophila* E-cadherin homolog) play an important role (Hozumi et al., 2006; Petzoldt et al., 2012; Speder et al., 2006), since asymmetric distribution of DE-cadherin dictates the coiling direction of the embryonic hindgut in *Drosophila* (Taniguchi et al., 2011).

Also, the work of Okumura and colleagues have identified *zipper*, which encodes a *Drosophila* non-muscle myosin II heavy chain, as an essential gene for the biased positioning of the embryonic anterior midgut (Okumura et al., 2010). They found myosin II was involved in the two major events in the LR patterning of the embryonic anterior midgut, concerning the biased positioning of the circular visceral muscle cells (that cover the midgut epithelium) and the rotation of the midgut itself. They have proposed that myosin II is responsible for the generation of force needed to lead to a LR biased morphogenesis (Okumura et al., 2010). Later, the work of González-Morales and colleagues showed that a molecular link between myoID and the Planar Cell Polarity atypical cadherin Dachsous (Ds). MyoID interacts with the intracellular domain of Ds, an essential link for the dextral polarity of neighboring hindgut progenitors and required for organ looping in *Drosophila* (Gonzalez-Morales et al., 2015). Taken together, it was shown in *Drosophila* that adherens junctions, myosin and PCP are important to connect LR asymmetry and cell and organ polarity. Also, it seems *Drosophila* has a unique mechanism to establish the LR through different organizers, since in vertebrates it is more or less established that a single developmental event is sufficient to determine LR patterning for all internal organs (Geminard et al., 2014).

6.2. Heart chiral looping

Another example of an internal organ whose formation is dependent on mechanical forces is the embryonic heart (Forouhar et al., 2006; Hove et al., 2003; Voronov et al., 2004) (Fig. 1B). Noel and colleagues, by using an *ex-vivo* heart culture system, have shown the dextral heart looping is a tissue-intrinsic process that requires the activity of actin and myosin (Noel et al., 2013). They have also demonstrated that *Nodal* signaling regulates *α-actin1b* gene expression asymmetricaly, suggesting that asymmetric *Nodal* signaling may enhance a cytoskeleton-based tissue-intrinsic mechanism of heart looping. Thus, this work supports the idea that chiral heart looping is a tissue-intrinsic process that could be controlled by both *Nodal*-dependent and -independent mechanisms (Noel et al., 2013).

Work done in the developing mouse heart (Linask et al., 2003; Linask et al., 2002; Lu et al., 2008) has shown that non-muscle myosin heavy chains IIA and IIB are asymmetrically expressed in the embryonic heart tube, and also, that their position seems to be strictly correlated with the direction of heart looping regardless of the expression of *Pitx2*. Moreover, when myosin-based tension generation is disrupted during the initial stages of heart looping, the whole process of cardiac morphogenesis is impaired (Wei et al., 2002). It does not seem that the constant variation of tension associated with heart contraction is important for heart looping as the heart still loops properly in the absence of contraction (Noel et al., 2013; Sehnert et al., 2002). Thus, it seems that tissue scale tension generated by differential cell shape is involved in providing the force for proper looping. Additional mechanical cues provided by the pericardial cavity might also be important (Bayraktar and Manner, 2014). Studies of heart looping biomechanics in chick embryos have produced interesting models to explain heart looping (Bayraktar and Manner, 2014; Shi et al., 2014). Bayraktar and Männer used a physical model to show that differential growth of the heart and pericardial cavity could contribute to a compressive load that provides extrinsic determinants for heart looping mechanics. Simulations of the growing heart tube constrained within the cavity buckle into a helical shape consistent with the shape of the c-looped heart tube (Bayraktar and Manner, 2014). In contrast to the "growth-induced buckling hypothesis" suggested by Bayraktar and Manner, Shi and colleagues proposed a model in which the differential hypertrophic growth of the myocardium acts as the main force responsible for bending the heart tube. Furthermore, they explore the fact that other regional growth and cytoskeletal contractions, as well as external compressive loads, drive the biased torsion of the heart tube. Here, the bending would be driven mainly by forces generated within the heart tube, while torsion would be caused by external loads (Shi et al., 2014). Even though biomechanical modeling of the embryonic heart is a powerful approach, there are still a number of parameters, such as the contribution of bending and torsion to the looping of the heart tube, that still remain difficult to assess experimentally making the current models difficult to validate.

6.3. Forces modulating branchial arch artery system asymmetry

Sometimes altered distribution of mechanical forces can provide surprising outcomes in the process of asymmetric development. The work of Yashiro and colleagues probably illustrate this the best. They showed that ablation of the unilateral asymmetric *Pitx2* expression impairs asymmetric remodeling of the branchial arch artery system, causing the aortic arch to develop with randomized laterality (Yashiro et al., 2007). They proposed a model in which *Pitx2* induces a regional morphological change that consequently generates an asymmetric blood flow in that region. The uneven distribution of blood flow induces a differential response of growth factors, leading to the maintenance of the left branchial arch artery and regression of its right counterpart, forming this way a left-sided aortic arch (Yashiro et al., 2007). Considering vascular morphogenesis is dependent on hemodynamics (Boselli et al., 2015; Freund et al., 2012), it is possible that the same flow responsive gene network involved in angiogenesis is at work in this process (Fig. 1B). In particular, it seems likely that a feedback loop involving stretch sensitive channels, such as Piezo 1 (Li et al., 2014; Ranade et al., 2014) could be involved in modulating endothelial cell response to forces and alter branchial arches morphogenesis. Interestingly, the homolog of klf2, a transcription factor whose expression is controlled by shear stress *in vivo* (Dekker et al., 2002; Lee et al., 2006), klf2a in zebrafish, has been shown to control branchial arches morphogenesis in response to flow forces (Nicoli et al., 2010).

7. Conclusion

It is now clear that mechanical forces constitute an essential element to include for our understanding of left-right patterning and asymmetric morphogenesis. At both the cellular or tissue scales, a number of unexpected asymmetric inputs can be provided by mechanical forces. They finally translate into asymmetric cell migration, directional flow mediated by beating cilia or chiral spreading of the cells. Together, it seems that mechanical forces can be used to balance or modulate the strong inputs of genetic signals in order to refine or reinforce the cell or tissue movements that are associated with asymmetric development. Not surprisingly, a number of these forces depend on cell contractility and actomyosin modulators. We predict that a lot will be gained from *in vitro* studies and biophysical studies aiming for the identification of the origins of the cellular chirality and how they are connected with the structural component of actomyosin proteins to generate the rotational forces at the base of the chiral organization of the actin network. Obviously, identifying the mechanism activating or inibiting chiral torque generation mediated by the actin cytoskeleton will be necessary to clarify the role of cytoskeletal chirality during left-right patterning and morphogenesis. We foresee key inputs coming from studies of self organising explants systems such as gastruloids, where axis patterning and nodal function start to be unravelled (Turner et al., 2016). Furthermore, quantifying the asymmetric forces generated within the LRO and during asymmetric morphogenesis will help to identify what are the relevant forces and what potential mechanosensors are involved. Finally, the array of mechanosensors at work in each system remain to be studied, and animal models like zebrafish, xenopus and chicken will help a lot in that quest because of their accessibility to experimentation and the possibility to directly impose forces on the embryo. Alternatives including organoids and the use 3D scaffolds should help in that quest as well (Clevers, 2016).

Acknowledgements

We thank Renée Chow and the Vermot lab for discussion and thoughtful comments on the manuscript. JV was supported by HFSP (CDA00032/2010-C), INSERM (Avenir 2008), AFM (14983 and 16852), FRM (DEQ20140329553), the Seventh Framework Program (MC-IRG256549), ANR (ANR-12-ISV2-0001, ANR-11-EQPX-0029, ANR-2010-JCJC-1510-01), EMBO YIP (2014), and by the grant ANR-10-LABX-0030-INRT, a French State fund managed by the Agence Nationale de la Recherche under the frame program Investissements d'Avenir labeled ANR-10-IDEX-0002-02. R.R.F. was supported by the IGBMC International PhD program (LABEX-INRT).

References

Anton, H., Harlepp, S., Ramspacher, C., Wu, D., Monduc, F., Bhat, S., Liebling, M., Paoletti, C., Charvin, G., Freund, J.B., et al., 2013. Pulse propagation by a capacitive mechanism drives embryonic blood flow. Development 140, 4426–4434.

Avasthi, P., Marshall, W.F., 2012. Stages of ciliogenesis and regulation of ciliary length. Differentiation 83, S30–S42.

Bayraktar, M., Manner, J., 2014. Cardiac looping may be driven by compressive loads resulting from unequal growth of the heart and pericardial cavity. Observations on a physical simulation model. Front. Physiol. 5, 112.

Behrndt, M., Salbreux, G., Campinho, P., Hauschild, R., Oswald, F., Roensch, J., Grill, S.W., Heisenberg, C.P., 2012. Forces driving epithelial spreading in zebrafish gastrulation. Science 338, 257–260.

Blum, M., Beyer, T., Weber, T., Vick, P., Andre, P., Bitzer, E., Schweickert, A., 2009. Xenopus, an ideal model system to study vertebrate left-right asymmetry. Dev. Dyn. 238, 1215–1225.

Blum, M., Feistel, K., Thumberger, T., Schweickert, A., 2014. The evolution and conservation of left-right patterning mechanisms. Development 141, 1603–1613.

Borovina, A., Superina, S., Voskas, D., Ciruna, B., 2010. Vangl2 directs the posterior tilting and asymmetric localization of motile primary cilia. Nat. Cell Biol. 12, 407–412.

Boselli, F., Freund, J.B., Vermot, J., 2015. Blood flow mechanics in cardiovascular development. Cell. Mol. Life Sci. 72, 2545–2559.

Brown, N.A., Wolpert, L., 1990. The development of handedness in left/right asymmetry. Development 109, 1–9.

Burdine, R.D., Schier, A.F., 2000. Conserved and divergent mechanisms in left-right axis formation. Genes Dev. 14, 763–776.

Caballero, D., Voituriez, R., Riveline, D., 2015. The cell ratchet: interplay between efficient protrusions and adhesion determines cell motion. Cell Adhes. Migr. 9, 327–334.

Capdevila, J., Vogan, K.J., Tabin, C.J., Izpisua Belmonte, J.C., 2000. Mechanisms of left-right determination in vertebrates. Cell 101, 9–21.

Cartwright, J.H., Piro, O., Tuval, I., 2009. Fluid dynamics in developmental biology: moving fluids that shape ontogeny. HFSP J. 3, 77–93.

Chen, T.H., Hsu, J.J., Zhao, X., Guo, C., Wong, M.N., Huang, Y., Li, Z., Garfinkel, A., Ho, C.M., Tintut, Y., et al., 2012. Left-right symmetry breaking in tissue morphogenesis via cytoskeletal mechanics. Circ. Res. 110, 551–559.

Chien, Y.H., Keller, R., Kintner, C., Shook, D.R., 2015. Mechanical strain determines the axis of planar polarity in ciliated epithelia. Curr. Biol. 25, 2774–2784.

Clevers, H., 2016. Modeling development and disease with organoids. Cell 165, 1586–1597.

Colantonio, J.R., Vermot, J., Wu, D., Langenbacher, A.D., Fraser, S., Chen, J.N., Hill, K.L., 2009. The dynein regulatory complex is required for ciliary motility and otolith biogenesis in the inner ear. Nature 457, 205–209.

Comelles, J., Caballero, D., Voituriez, R., Hortiguela, V., Wollrab, V., Godeau, A.L., Samitier, J., Martinez, E., Riveline, D., 2014. Cells as active particles in asymmetric potentials: motility under external gradients. Biophys. J. 107, 1513–1522.

Compagnon, J., Barone, V., Rajshekar, S., Kottmeier, R., Pranjic-Ferscha, K., Behrndt, M., Heisenberg, C.P., 2014. The notochord breaks bilateral symmetry by controlling cell shapes in the zebrafish laterality organ. Dev. Cell 31, 774–783.

Cui, C., Little, C.D., Rongish, B.J., 2009. Rotation of organizer tissue contributes to left-right asymmetry. Anat. Rec. (Hoboken) 292, 557–561.

Davis, N.M., Kurpios, N.A., Sun, X., Gros, J., Martin, J.F., Tabin, C.J., 2008. The chirality of gut rotation derives from left-right asymmetric changes in the architecture of the dorsal mesentery. Dev. Cell 15, 134–145.

Dekker, R.J., van Soest, S., Fontijn, R.D., Salamanca, S., de Groot, P.G., VanBavel, E., Pannekoek, H., Horrevoets, A.J., 2002. Prolonged fluid shear stress induces a distinct set of endothelial cell genes, most specifically lung Kruppel-like factor (KLF2). Blood 100, 1689–1698.

Delling, M., Indzhykulian, A.A., Liu, X., Li, Y., Xie, T., Corey, D.P., Clapham, D.E., 2016. Primary cilia are not calcium-responsive mechanosensors. Nature 531, 656–660.

Delmas, P., Nauli, S.M., Li, X., Coste, B., Osorio, N., Crest, M., Brown, D.A., Zhou, J., 2004. Gating of the polycystin ion channel signaling complex in neurons and kidney cells. FASEB J. 18, 740–742.

Du, J., Ma, X., Shen, B., Huang, Y., Birnbaumer, L., Yao, X., 2014. TRPV4, TRPC1, and TRPP2 assemble to form a flow-sensitive heteromeric channel. FASEB J. 28, 4677–4685.

Essner, J.J., Amack, J.D., Nyholm, M.K., Harris, E.B., Yost, H.J., 2005. Kupffer's vesicle is a ciliated organ of asymmetry in the zebrafish embryo that initiates left-right development of the brain, heart and gut. Development 132, 1247–1260.

Farge, E., 2003. Mechanical induction of twist in the *Drosophila* foregut/stomodeal primordium. Curr. Biol. 13, 1365–1377.

Farina, F., Gaillard, J., Guerin, C., Coute, Y., Sillibourne, J., Blanchoin, L., Thery, M., 2016. The centrosome is an actin-organizing centre. Nat. Cell Biol. 18 (65-+).

Fernandez-Sanchez, M.E., Serman, F., Ahmadi, P., Farge, E., 2010. Mechanical induction in embryonic development and tumor growth integrative cues through molecular to multicellular interplay and evolutionary perspectives. Methods Cell Biol. 98, 295–321.

Fernandez-Sanchez, M.E., Barbier, S., Whitehead, J., Bealle, G., Michel, A., Latorre-Ossa, H., Rey, C., Fouassier, L., Claperon, A., Brulle, L., et al., 2015. Mechanical induction of the tumorigenic beta-catenin pathway by tumour growth pressure. Nature 523, 92–95.

Field, S., Riley, K.L., Grimes, D.T., Hilton, H., Simon, M., Powles-Glover, N., Siggers, P., Bogani, D., Greenfield, A., Norris, D.P., 2011. Pkd1l1 establishes left-right asymmetry and physically interacts with Pkd2. Development 138, 1131–1142.

Forouhar, A.S., Liebling, M., Hickerson, A., Nasiraei-Moghaddam, A., Tsai, H.J., Hove, J.R., Fraser, S.E., Dickinson, M.E., Gharib, M., 2006. The embryonic vertebrate heart tube is a dynamic suction pump. Science 312, 751–753.

Freund, J.B., Goetz, J.G., Hill, K.L., Vermot, J., 2012. Fluid flows and forces in development: functions, features and biophysical principles. Development 139, 1229–1245.

Garcia-Castro, M.I., Vielmetter, E., Bronner-Fraser, M., 2000. N-cadherin, a cell adhesion molecule involved in establishment of embryonic left-right asymmetry. Science 288, 1047–1051.

Geminard, C., Gonzalez-Morales, N., Coutelis, J.B., Noselli, S., 2014. The myosin ID pathway and left-right asymmetry in *Drosophila*. Genesis 52, 471–480.

Giamarchi, A., Padilla, F., Coste, B., Raoux, M., Crest, M., Honore, E., Delmas, P., 2006. The versatile nature of the calcium-permeable cation channel TRPP2. EMBO Rep. 7, 787–793.

Goetz, J.G., Steed, E., Ferreira, R.R., Roth, S., Ramspacher, C., Boselli, F., Charvin, G., Liebling, M., Wyart, C., Schwab, Y., et al., 2014. Endothelial cilia mediate low flow sensing during zebrafish vascular development. Cell Rep. 6, 799–808.

Gonzalez-Morales, N., Geminard, C., Lebreton, G., Cerezo, D., Coutelis, J.B., Noselli, S., 2015. The atypical cadherin Dachsous controls left-right asymmetry in *Drosophila*. Dev. Cell 33, 675–689.

Grill, S.W., 2011. Growing up is stressful: biophysical laws of morphogenesis. Curr. Opin. Genet. Dev. 21, 647–652.

Grimes, D.T., Keynton, J.L., Buenavista, M.T., Jin, X., Patel, S.H., Kyosuke, S., Vibert, J., Williams, D.J., Hamada, H., Hussain, R., et al., 2016. Genetic analysis reveals a hierarchy of interactions between polycystin-encoding genes and genes controlling cilia function during left-right determination. PLoS Genet. 12, e1006070.

Gros, J., Feistel, K., Viebahn, C., Blum, M., Tabin, C.J., 2009. Cell movements at Hensen's node establish left/right asymmetric gene expression in the chick. Science 324, 941–944.

Guirao, B., Meunier, A., Mortaud, S., Aguilar, A., Corsi, J.M., Strehl, L., Hirota, Y., Desoeuvre, A., Boutin, C., Han, Y.G., et al., 2010. Coupling between hydrodynamic forces and planar cell polarity orients mammalian motile cilia. Nat. Cell Biol. 12, 341–350.

Hahn, C., Schwartz, M.A., 2009. Mechanotransduction in vascular physiology and atherogenesis. Nat. Rev. Mol. Cell Biol. 10, 53–62.

Hamada, H., 2015. Role of physical forces in embryonic development. Semin. Cell Dev. Biol. 47-48, 88–91.

Hamada, H., Meno, C., Watanabe, D., Saijoh, Y., 2002. Establishment of vertebrate left-right asymmetry. Nat. Rev. Genet. 3, 103–113.

Hanaoka, K., Qian, F., Boletta, A., Bhumia, A.K., Piontek, K., Tsiokas, L., Sukhatme, V.P., Guggino, W.B., Germino, G.G., 2000. Co-assembly of polycystin-1 and-2 produces unique cation-permeable currents. Nature 408, 990–994.

Hashimoto, M., Hamada, H., 2010. Translation of anterior-posterior polarity into left-right polarity in the mouse embryo. Curr. Opin. Genet. Dev. 20, 433–437.

Hashimoto, M., Shinohara, K., Wang, J., Ikeuchi, S., Yoshiba, S., Meno, C., Nonaka, S., Takada, S., Hatta, K., Wynshaw-Boris, A., et al., 2010. Planar polarization of node cells determines the rotational axis of node cilia. Nat. Cell Biol. 12, 170–176.

Heckel, E., Boselli, F., Roth, S., Krudewig, A., Belting, H.G., Charvin, G., Vermot, J., 2015. Oscillatory flow modulates mechanosensitive klf2a expression through trpv4 and trpp2 during heart valve development. Curr. Biol. 25, 1354–1361.

Heisenberg, C.P., Bellaiche, Y., 2013. Forces in tissue morphogenesis and patterning. Cell 153, 948–962.

Hildebrandt, F., Benzing, T., Katsanis, N., 2011. Ciliopathies. N. Engl. J. Med. 364, 1533–1543.

Hilfinger, A., Julicher, F., 2008. The chirality of ciliary beats. Phys. Biol. 5, 016003.

Hiramatsu, R., Matsuoka, T., Kimura-Yoshida, C., Han, S.W., Mochida, K., Adachi, T., Takayama, S., Matsuo, I., 2013. External mechanical cues trigger the establishment of the anterior-posterior axis in early mouse embryos. Dev. Cell 27, 131–144.

Hochgreb-Hagele, T., Yin, C., Koo, D.E., Bronner, M.E., Stainier, D.Y., 2013. Laminin beta1a controls distinct steps during the establishment of digestive organ laterality. Development 140, 2734–2745.

Horne-Badovinac, S., Rebagliati, M., Stainier, D.Y., 2003. A cellular framework for gut-looping morphogenesis in zebrafish. Science 302, 662–665.

Hove, J.R., Koster, R.W., Forouhar, A.S., Acevedo-Bolton, G., Fraser, S.E., Gharib, M., 2003. Intracardiac fluid forces are an essential epigenetic factor for embryonic cardiogenesis. Nature 421, 172–177.

Hozumi, S., Maeda, R., Taniguchi, K., Kanai, M., Shirakabe, S., Sasamura, T., Speder, P., Noselli, S., Aigaki, T., Murakami, R., et al., 2006. An unconventional myosin in *Drosophila* reverses the default handedness in visceral organs. Nature 440, 798–802.

Huveneers, S., de Rooij, J., 2013. Mechanosensitive systems at the cadherin-F-actin interface. J. Cell Sci. 126, 403–413.

Ishikawa, H., Marshall, W.F., 2014. Mechanobiology of ciliogenesis. Bioscience 64, 1084–1091.

Kamura, K., Kobayashi, D., Uehara, Y., Koshida, S., Iijima, N., Kudo, A., Yokoyama, T., Takeda, H., 2011. Pkd1l1 complexes with Pkd2 on motile cilia and functions to establish the left-right axis. Development 138, 1121–1129.

Keeling, J., Tsiokas, L., Maskey, D., 2016. Cellular mechanisms of ciliary length control. Cell 5.

Kim, S., Nie, H., Nesin, V., Tran, U., Outeda, P., Bai, C.X., Keeling, J., Maskey, D., Watnick, T., Wessely, O., et al., 2016. The polycystin complex mediates Wnt/Ca(2+) signalling. Nat. Cell Biol. 18, 752–764.

Kramer-Zucker, A.G., Olale, F., Haycraft, C.J., Yoder, B.K., Schier, A.F., Drummond, I.A., 2005. Cilia-driven fluid flow in the zebrafish pronephros, brain and Kupffer's vesicle is required for normal organogenesis. Development 132, 1907–1921.

Kreiling, J.A., Prabhat Williams, G., Creton, R., 2007. Analysis of Kupffer's vesicle in zebrafish embryos using a cave automated virtual environment. Dev. Dyn. 236, 1963–1969.

Kurpios, N.A., Ibanes, M., Davis, N.M., Lui, W., Katz, T., Martin, J.F., Izpisua Belmonte, J.C., Tabin, C.J., 2008. The direction of gut looping is established by changes in the extracellular matrix and in cell:cell adhesion. Proc. Natl. Acad. Sci. U. S. A. 105, 8499–8506.

Ladoux, B., Anon, E., Lambert, M., Rabodzey, A., Hersen, P., Buguin, A., Silberzan, P., Mege, R.M., 2010. Strength dependence of cadherin-mediated adhesions. Biophys. J. 98, 534–542.

Ladoux, B., Mege, R.M., Trepat, X., 2016. Front-rear polarization by mechanical cues: from single cells to tissues. Trends Cell Biol. 26, 420–433.

Lecuit, T., Yap, A.S., 2015. E-cadherin junctions as active mechanical integrators in tissue dynamics. Nat. Cell Biol. 17, 533–539.

Lecuit, T., Lenne, P.F., Munro, E., 2011. Force generation, transmission, and integration during cell and tissue morphogenesis. Annu. Rev. Cell Dev. Biol. 27, 157–184.

Lee, J.S., Yu, Q., Shin, J.T., Sebzda, E., Bertozzi, C., Chen, M., Mericko, P., Stadtfeld, M., Zhou, D., Cheng, L., et al., 2006. Klf2 is an essential regulator of vascular hemodynamic forces in vivo. Dev. Cell 11, 845–857.

LeGoff, L., Lecuit, T., 2016. Mechanical forces and growth in animal tissues. Cold Spring Harb. Perspect. Biol. 8, a019232.

Levin, M., 2005. Left-right asymmetry in embryonic development: a comprehensive review. Mech. Dev. 122, 3–25.

Levin, M., Mercola, M., 1998. The compulsion of chirality: toward an understanding of left-right asymmetry. Genes Dev. 12, 763–769.

Li, J., Hou, B., Tumova, S., Muraki, K., Bruns, A., Ludlow, M.J., Sedo, A., Hyman, A.J., McKeown, L., Young, R.S., et al., 2014. Piezo1 integration of vascular architecture with physiological force. Nature 515, 279–282.

Linask, K.K., Yu, X., Chen, Y., Han, M.D., 2002. Directionality of heart looping: effects of Pitx2c misexpression on flectin asymmetry and midline structures. Dev. Biol. 246, 407–417.

Linask, K.K., Han, M.D., Linask, K.L., Schlange, T., Brand, T., 2003. Effects of antisense misexpression of CFC on downstream flectin protein expression during heart looping. Dev. Dyn. 228, 217–230.

Lu, W., Seeholzer, S.H., Han, M., Arnold, A.S., Serrano, M., Garita, B., Philp, N.J., Farthing, C., Steele, P., Chen, J., et al., 2008. Cellular nonmuscle myosins NMHC-IIA and NMHC-IIB and vertebrate heart looping. Dev. Dyn. 237, 3577–3590.

Mahadevan, A., Welsh, I.C., Sivakumar, A., Gludish, D.W., Shilvock, A.R., Noden, D.M., Huss, D., Lansford, R., Kurpios, N.A., 2014. The left-right Pitx2 pathway drives organ-specific arterial and lymphatic development in the intestine. Dev. Cell 31, 690–706.

Maitre, J.L., Berthoumieux, H., Krens, S.F., Salbreux, G., Julicher, F., Paluch, E., Heisenberg, C.P., 2012. Adhesion functions in cell sorting by mechanically coupling the cortices of adhering cells. Science 338, 253–256.

Mammoto, T., Ingber, D.E., 2010. Mechanical control of tissue and organ development. Development 137, 1407–1420.

Mammoto, A., Mammoto, T., Ingber, D.E., 2012. Mechanosensitive mechanisms in transcriptional regulation. J. Cell Sci. 125, 3061–3073.

McDowell, G., Rajadurai, S., Levin, M., 2016. From cytoskeletal dynamics to organ asymmetry: a nonlinear, regulative pathway underlies left-right patterning. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 371.

McGrath, J., Somlo, S., Makova, S., Tian, X., Brueckner, M., 2003. Two populations of node monocilia initiate left-right asymmetry in the mouse. Cell 114, 61–73.

Mendes, R.V., Martins, G.G., Cristovao, A.M., Saude, L., 2014. N-cadherin locks left-right asymmetry by ending the leftward movement of Hensen's node cells. Dev. Cell 30, 353–360.

Mirabet, V., Das, P., Boudaoud, A., Hamant, O., 2011. The role of mechanical forces in plant morphogenesis. Annu. Rev. Plant Biol. 62, 365–385.

Naganathan, S.R., Furthauer, S., Nishikawa, M., Julicher, F., Grill, S.W., 2014. Active torque generation by the actomyosin cell cortex drives left-right symmetry breaking. Elife 3, e04165.

Naganathan, S.R., Middelkoop, T.C., Furthauer, S., Grill, S.W., 2016. Actomyosin-driven left-right asymmetry: from molecular torques to chiral self-organization. Curr. Opin. Cell Biol. 38, 24–30.

Nakamura, T., Mine, N., Nakaguchi, E., Mochizuki, A., Yamamoto, M., Yashiro, K., Meno, C., Hamada, H., 2006. Generation of robust left-right asymmetry in the mouse embryo requires a self-enhancement and lateral-inhibition system. Dev. Cell 11, 495–504.

Nauli, S.M., Alenghat, F.J., Luo, Y., Williams, E., Vassilev, P., Li, X., Elia, A.E., Lu, W., Brown, E.M., Quinn, S.J., et al., 2003. Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. Nat. Genet. 33, 129–137.

Nauli, S.M., Kawanabe, Y., Kaminski, J.J., Pearce, W.J., Ingber, D.E., Zhou, J., 2008. Endothelial cilia are fluid shear sensors that regulate calcium signaling and nitric oxide production through polycystin-1. Circulation 117, 1161–1171.

Ng, A.N., de Jong-Curtain, T.A., Mawdsley, D.J., White, S.J., Shin, J., Appel, B., Dong, P.D., Stainier, D.Y., Heath, J.K., 2005. Formation of the digestive system in zebrafish: III. Intestinal epithelium morphogenesis. Dev. Biol. 286, 114–135.

Nicoli, S., Standley, C., Walker, P., Hurlstone, A., Fogarty, K.E., Lawson, N.D., 2010. MicroRNA-mediated integration of haemodynamics and Vegf signalling during angiogenesis. Nature 464, 1196–1200.

Noel, E.S., Verhoeven, M., Lagendijk, A.K., Tessadori, F., Smith, K., Choorapoikayil, S., den Hertog, J., Bakkers, J., 2013. A nodal-independent and tissue-intrinsic mechanism controls heart-looping chirality. Nat. Commun. 4, 2754.

Nonaka, S., Tanaka, Y., Okada, Y., Takeda, S., Harada, A., Kanai, Y., Kido, M., Hirokawa, N., 1998. Randomization of left-right asymmetry due to loss of nodal cilia generating leftward flow of extraembryonic fluid in mice lacking KIF3B motor protein. Cell 95, 829–837.

Ober, E.A., Field, H.A., Stainier, D.Y., 2003. From endoderm formation to liver and pancreas development in zebrafish. Mech. Dev. 120, 5–18.

Ohata, S., Herranz-Perez, V., Nakatani, J., Boletta, A., Garcia-Verdugo, J.M., Alvarez-Buylla, A., 2015. Mechanosensory genes Pkd1 and Pkd2 contribute to the planar polarization of brain ventricular epithelium. J. Neurosci. 35, 11153–11168.

Okabe, N., Xu, B., Burdine, R.D., 2008. Fluid dynamics in zebrafish Kupffer's vesicle. Dev. Dyn. 237, 3602–3612.

Okada, Y., Takeda, S., Tanaka, Y., Izpisua Belmonte, J.C., Hirokawa, N., 2005. Mechanism of nodal flow: a conserved symmetry breaking event in left-right axis determination. Cell 121, 633–644.

Okumura, T., Fujiwara, H., Taniguchi, K., Kuroda, J., Nakazawa, N., Nakamura, M., Hatori, R., Ishio, A., Maeda, R., Matsuno, K., 2010. Left-right asymmetric morphogenesis of the anterior midgut depends on the activation of a non-muscle myosin II in *Drosophila*. Dev. Biol. 344, 693–706.

Pardanaud, L., Eichmann, A., 2009. Stem cells: the stress of forming blood cells. Nature 459, 1068–1069.

Patel, A., Sharif-Naeini, R., Folgering, J.R., Bichet, D., Duprat, F., Honore, E., 2010. Canonical TRP channels and mechanotransduction: from physiology to disease states. Pflugers Arch. 460, 571–581.

Pazour, G.J., San Agustin, J.T., Follit, J.A., Rosenbaum, J.L., Witman, G.B., 2002. Polycystin-2 localizes to kidney cilia and the ciliary level is elevated in orpk mice with polycystic kidney disease. Curr. Biol. 12, R378–R380.

Pennekamp, P., Karcher, C., Fischer, A., Schweickert, A., Skryabin, B., Horst, J., Blum, M., Dworniczak, B., 2002. The ion channel polycystin-2 is required for left-right axis determination in mice. Curr. Biol. 12, 938–943.

Pennekamp, P., Menchen, T., Dworniczak, B., Hamada, H., 2015. Situs inversus and ciliary abnormalities: 20 years later, what is the connection? Cilia 4, 1.

Peralta, M., Steed, E., Harlepp, S., Gonzalez-Rosa, J.M., Monduc, F., Ariza-Cosano, A., Cortes, A., Rayon, T., Gomez-Skarmeta, J.L., Zapata, A., et al., 2013. Heartbeat-driven pericardiac fluid forces contribute to epicardium morphogenesis. Curr. Biol. 23, 1726–1735.

Petzoldt, A.G., Coutelis, J.B., Geminard, C., Speder, P., Suzanne, M., Cerezo, D., Noselli, S., 2012. DE-cadherin regulates unconventional myosin ID and myosin IC in *Drosophila* left-right asymmetry establishment. Development 139, 1874–1884.

Pitaval, A., Tseng, Q., Bornens, M., Thery, M., 2010. Cell shape and contractility regulate ciliogenesis in cell cycle-arrested cells. J. Cell Biol. 191, 303–312.

Pohl, C., Bao, Z., 2010. Chiral forces organize left-right patterning in *C. elegans* by uncoupling midline and anteroposterior axis. Dev. Cell 19, 402–412.

Pourquie, O., 2011. Vertebrate segmentation: from cyclic gene networks to scoliosis. Cell 145, 650–663.

Ranade, S.S., Qiu, Z., Woo, S.H., Hur, S.S., Murthy, S.E., Cahalan, S.M., Xu, J., Mathur, J., Bandell, M., Coste, B., et al., 2014. Piezo1, a mechanically activated ion channel, is required for vascular development in mice. Proc. Natl. Acad. Sci. U. S. A. 111, 10347–10352.

Sampaio, P., Ferreira, R.R., Guerrero, A., Pintado, P., Tavares, B., Amaro, J., Smith, A.A., Montenegro-Johnson, T., Smith, D.J., Lopes, S.S., 2014. Left-right organizer flow dynamics: how much cilia activity reliably yields laterality? Dev. Cell 29, 716–728.

Savin, T., Kurpios, N.A., Shyer, A.E., Florescu, P., Liang, H., Mahadevan, L., Tabin, C.J., 2011. On the growth and form of the gut. Nature 476, 57–62.

Sawamoto, K., Wichterle, H., Gonzalez-Perez, O., Cholfin, J.A., Yamada, M., Spassky, N., Murcia, N.S., Garcia-Verdugo, J.M., Marin, O., Rubenstein, J.L., et al., 2006. New neurons follow the flow of cerebrospinal fluid in the adult brain. Science 311, 629–632.

Schonegg, S., Hyman, A.A., Wood, W.B., 2014. Timing and mechanism of the initial cue establishing handed left-right asymmetry in *Caenorhabditis elegans* embryos. Genesis 52, 572–580.

Schottenfeld, J., Sullivan-Brown, J., Burdine, R.D., 2007. Zebrafish curly up encodes a Pkd2 ortholog that restricts left-side-specific expression of southpaw. Development 134, 1605–1615.

Schweickert, A., Weber, T., Beyer, T., Vick, P., Bogusch, S., Feistel, K., Blum, M., 2007. Cilia-driven leftward flow determines laterality in *Xenopus*. Curr. Biol. 17, 60–66.

Sehnert, A.J., Huq, A., Weinstein, B.M., Walker, C., Fishman, M., Stainier, D.Y., 2002. Cardiac troponin T is essential in sarcomere assembly and cardiac contractility. Nat. Genet. 31, 106–110.

Sharif-Naeini, R., Folgering, J.H., Bichet, D., Duprat, F., Lauritzen, I., Arhatte, M., Jodar, M., Dedman, A., Chatelain, F.C., Schulte, U., et al., 2009. Polycystin-1 and -2 dosage regulates pressure sensing. Cell 139, 587–596.

Sharif-Naeini, R., Folgering, J.H., Bichet, D., Duprat, F., Delmas, P., Patel, A., Honore, E., 2010. Sensing pressure in the cardiovascular system: Gq-coupled mechanoreceptors and TRP channels. J. Mol. Cell. Cardiol. 48, 83–89.

Shi, Y., Yao, J., Young, J.M., Fee, J.A., Perucchio, R., Taber, L.A., 2014. Bending and twisting the embryonic heart: a computational model for c-looping based on realistic geometry. Front. Physiol. 5, 297.

Shinohara, K., Kawasumi, A., Takamatsu, A., Yoshiba, S., Botilde, Y., Motoyama, N., Reith, W., Durand, B., Shiratori, H., Hamada, H., 2012. Two rotating cilia in the node cavity are sufficient to break left-right symmetry in the mouse embryo. Nat. Commun. 3, 622.

Singh, D., Pohl, C., 2014. Coupling of rotational cortical flow, asymmetric midbody positioning, and spindle rotation mediates dorsoventral axis formation in *C. elegans*. Dev. Cell 28, 253–267.

Song, H., Hu, J., Chen, W., Elliott, G., Andre, P., Gao, B., Yang, Y., 2010. Planar cell polarity breaks bilateral symmetry by controlling ciliary positioning. Nature 466, 378–382.

Speder, P., Noselli, S., 2007. Left-right asymmetry: class I myosins show the direction. Curr. Opin. Cell Biol. 19, 82–87.

Speder, P., Adam, G., Noselli, S., 2006. Type ID unconventional myosin controls left-right asymmetry in *Drosophila*. Nature 440, 803–807.

Striedter, G.F., Srinivasan, S., Monuki, E.S., 2015. Cortical folding: when, where, how, and why? Annu. Rev. Neurosci. 38:291–307. http://dx.doi.org/10.1146/annurev-neuro-071714-034128 (Jul. 8).

Sugimura, K., Lenne, P.F., Graner, F., 2016. Measuring forces and stresses in situ in living tissues. Development 143, 186–196.

Supatto, W., Vermot, J., 2011. From cilia hydrodynamics to zebrafish embryonic development. Curr. Top. Dev. Biol. 95, 33–66.

Superina, S., Borovina, A., Ciruna, B., 2014. Analysis of maternal-zygotic ugdh mutants reveals divergent roles for HSPGs in vertebrate embryogenesis and provides new insight into the initiation of left-right asymmetry. Dev. Biol. 387, 154–166.

Tabin, C.J., Vogan, K.J., 2003. A two-cilia model for vertebrate left-right axis specification. Genes Dev. 17, 1–6.

Taniguchi, K., Maeda, R., Ando, T., Okumura, T., Nakazawa, N., Hatori, R., Nakamura, M., Hozumi, S., Fujiwara, H., Matsuno, K., 2011. Chirality in planar cell shape contributes to left-right asymmetric epithelial morphogenesis. Science 333, 339–341.

Tee, Y.H., Shemesh, T., Thiagarajan, V., Hariadi, R.F., Anderson, K.L., Page, C., Volkmann, N., Hanein, D., Sivaramakrishnan, S., Kozlov, M.M., et al., 2015. Cellular chirality arising from the self-organization of the actin cytoskeleton. Nat. Cell Biol. 17, 445–457.

Turner, D.A., Glodowski, C.R., Luz, A.C., Baillie-Johnson, P., Hayward, P.C., Collignon, J., Gustavsen, C., Serup, P., Schröter, C., Martinez Arias, A., 2016. Interactions between Nodal and Wnt signalling Drive Robust Symmetry Breaking and Axial Organisation in Gastruloids (Embryonic Organoids). bioRxiv, 051722 http://dx.doi.org/10.1101/051722.

Vincensini, L., Blisnick, T., Bastin, P., 2011. 1001 model organisms to study cilia and flagella. Biol. Cell. 103, 109–130.
Vogel, V., Sheetz, M., 2006. Local force and geometry sensing regulate cell functions. Nat. Rev. Mol. Cell Biol. 7, 265–275.
Voronov, D.A., Alford, P.W., Xu, G., Taber, L.A., 2004. The role of mechanical forces in dextral rotation during cardiac looping in the chick embryo. Dev. Biol. 272, 339–350.
Wallace, K.N., Pack, M., 2003. Unique and conserved aspects of gut development in zebrafish. Dev. Biol. 255, 12–29.
Wang, G., Cadwallader, A.B., Jang, D.S., Tsang, M., Yost, H.J., Amack, J.D., 2011. The Rho kinase Rock2b establishes anteroposterior asymmetry of the ciliated Kupffer's vesicle in zebrafish. Development 138, 45–54.
Wang, G., Manning, M.L., Amack, J.D., 2012. Regional cell shape changes control form and function of Kupffer's vesicle in the zebrafish embryo. Dev. Biol. 370, 52–62.
Weber, G.F., Bjerke, M.A., DeSimone, D.W., 2012. A mechanoresponsive cadherin-keratin complex directs polarized protrusive behavior and collective cell migration. Dev. Cell 22, 104–115.
Wei, L., Imanaka-Yoshida, K., Wang, L., Zhan, S., Schneider, M.D., DeMayo, F.J., Schwartz, R.J., 2002. Inhibition of Rho family GTPases by Rho GDP dissociation inhibitor disrupts cardiac morphogenesis and inhibits cardiomyocyte proliferation. Development 129, 1705–1714.
Welsh, I.C., Thomsen, M., Gludish, D.W., Alfonso-Parra, C., Bai, Y., Martin, J.F., Kurpios, N.A., 2013. Integration of left-right Pitx2 transcription and Wnt signaling drives asymmetric gut morphogenesis via Daam2. Dev. Cell 26, 629–644.
White, J.P., Cibelli, M., Urban, L., Nilius, B., McGeown, J.G., Nagy, I., 2016. TRPV4: molecular conductor of a diverse orchestra. Physiol. Rev. 96, 911–973.
Wu, D., Freund, J.B., Fraser, S.E., Vermot, J., 2011. Mechanistic basis of otolith formation during teleost inner ear development. Dev. Cell 20, 271–278.
Yashiro, K., Shiratori, H., Hamada, H., 2007. Haemodynamics determined by a genetic programme govern asymmetric development of the aortic arch. Nature 450, 285–288.
Yin, C., Kikuchi, K., Hochgreb, T., Poss, K.D., Stainier, D.Y., 2010. Hand2 regulates extracellular matrix remodeling essential for gut-looping morphogenesis in zebrafish. Dev. Cell 18, 973–984.
Yoder, B.K., Hou, X., Guay-Woodford, L.M., 2002. The polycystic kidney disease proteins, polycystin-1, polycystin-2, polaris, and cystin, are co-localized in renal cilia. J. Am. Soc. Nephrol. 13, 2508–2516.
Yoshiba, S., Shiratori, H., Kuo, I.Y., Kawasumi, A., Shinohara, K., Nonaka, S., Asai, Y., Sasaki, G., Belo, J.A., Sasaki, H., et al., 2012. Cilia at the node of mouse embryos sense fluid flow for left-right determination via Pkd2. Science 338, 226–231.
Yuan, S., Zhao, L., Brueckner, M., Sun, Z., 2015. Intraciliary calcium oscillations initiate vertebrate left-right asymmetry. Curr. Biol. 25, 556–567.
Zhang, H., Labouesse, M., 2012. Signalling through mechanical inputs: a coordinated process. J. Cell Sci. 125, 3039–3049.
Zhang, H., Gally, C., Labouesse, M., 2010. Tissue morphogenesis: how multiple cells cooperate to generate a tissue. Curr. Opin. Cell Biol. 22, 575–582.